Introduction

Estuarine environment is a unique system where there are spatial and temporal variations in environmental parameters which range from fresh water to marine conditions. Estuarine sediment and overlying water interact with each other and collectively contribute this unique environment to the organisms. The environmental parameters have got great influence on the species diversity, biomass and population density of soft bottom macro-fauna. It is essential to gather information on the environmental factors influencing, directly or indirectly, on the distribution and density of estuarine macrobenthos. Most of the Venerid clams are ecologically important and commercially valuable. The study of phylogenetic relationship among Venerid clams is complicated by their morphological parallelism among evolutionary distant species and by shell diversification among closely related species. Because of great diversity and the lack of systematic studies, venus clams have a confusing taxonomic history and their interrelationships remain poorly understood. Present study focuses on the adaptability, distribution status and phylogeny of two Venerid clams, *Paphia malabarica* and *Meretrix casta* in two estuaries of Kerala, Ashtamudi estuary and Kayamkulam estuary.

Objectives of the study

Major objectives of the study are to analyse.

1. Systematics, distribution and adaptability of two Venerid clams *Paphia malabarica* and *Meretrix casta.*

2. Hydrographic parameters of estuary and its impact on clams.

3. Sediment parameters of the clam beds and its impact on clams.

4. Morphometry and allometry of the two clam species.

5. Inferring phylogenetic relationship between the two clams through molecular data

Methodology

Monthly samples of bottom water and sediment were collected from the sampling sites of two estuarine systems.

Hydrologic parameters studied

Water samples were analysed for salinity (Mohr-Knudsen method), dissolved oxygen (modified Winklers method), p^H (electrometric method) and temperature.

Sediment parameters studied

Sediment samples were used for determining sediment texture (pipette analysis), electrical conductivity (electrometric method), sediment p^H (electrometric method), organic carbon (Walkley-Black method) and sediment nutrients such as phosphorus (sodium-bicarbonate method) and potassium (flame photometric method).

Clam density and distribution were determined using Quadrate (0.25 m^2) method.

Statistical analyses were carried out to find the correlation between clam density and environmental variables. Significant environmental variables influencing the density and distribution of selected clams in the estuaries were determined through Principal Component Analysis (PCA). Environmental and biological variables were subjected to ANOVA and post hoc tests.

Venerid clams *Paphia malabarica* and *Meretrix casta* were collected for biometric measurements (morphometric/allometric) and molecular analysis. Morphometric trait analysis (Landmark-based method) was carried out. t-test was conducted to evaluate species-specific differences in morphometric traits.

Allometric relationships between biometric variables such as total shell length (L), shell height (H), total weight (TW), visceral mass weight (VW) and shell weight (SW) were made by adjustment of a linear function of data. Determination coefficient (r^2) was calculated to determine the association between variables. All statistical analyses were carried out using Statistical Package for Social Sciences (SPSS 20.0 Version).

Adult clam samples of *Paphia malabarica* and *Meretrix casta* were used for molecular analyses. Total genomic DNA was extracted following modified CTAB protocol (Winnepenninckx *et al*., 1993). Using ITS1 and ITS2 forward and reverse primers (Primer Select Software of DNA Star Package Version 5.01), internal transcribed spacer region of nuclear ribosomal DNA was amplified. The forward and reverse sequences were assembled using SeqManII software in DNA Star Package to obtain ITS1 and ITS2 sequences. Obtained ITS1, 5.8s, ITS2, 28s sequences were submitted in NCBI GenBank. The boundaries of coding and spacer regions were determined by comparison with sequence information of *Arctica islandica* (GenBank accession No.AF202106). The sequences were edited and analysed using Programme EdiSeq. ITS sequences containing the 5.8s DNA in two species

were aligned using ClustalW method. The phylogenetic trees were produced by Neighbour-Joining (NJ), Maximum Parsimony (MP), Minimum evolution (ME) and Unweighted Pair Group Method with Arithmetic Mean (UPGMA) methods using MEGA Software Version 4 (Tamura *et al*., 2007).

Results

Hydrologic parameters except water p^H exhibited significant spatio-temporal variations in Ashtamudi estuary. Salinity and temperature exhibited significant spatio-temporal variations in Kayamkulam estuary. Except water p^H all other physico-chemical parameters of two estuarine systems exhibited similar trend. Sediment parameters exhibited significant spatio-temporal variations in both estuaries. No seasonal variations were exhibited by sediment texture in Ashtamudi estuary. Sediment texture of Kayamkulam estuary exhibited spatio-temporal variations. Sediment p^H remained predominantly acidic throughout the study period. The values of sediment electrical conductivity and nutrients such as phosphorus and potassium showed significant variations between estuaries.

The density and distribution of focal species was not static during the study period in both the estuaries. The density and distribution of clams exhibited spatio-temporal variations in estuaries as well as between two estuaries. The Principal Component Analysis (PCA) result confirms the impact of multiple environmental factors on the density and distribution of estuarine bivalves. Factors such as sand fraction of sediment, salinity, dissolved oxygen, temperature and p^H were found to be influencing clam density and distribution. *Paphia malabarica*, being a burrowing bivalve, its density and distribution was impacted by salinity, sediment nutrients and organic carbon content. *Meretrix casta,* being an epibenthic clam , its abundance and distribution was greatly influenced by dissolved oxygen and sand fraction of sediment.

Morphometric trait analysis supported significant differences in shell shape between inter-species and intra-species clam populations. The external shell trait analysis revealed phenotypic plasticity in *Paphia malabarica* of Ashtamudi estuary. The inter-population differences of two clam species between two estuaries were also significant. In both the clam species the populations of Kayamkulam estuary were found to be slightly larger than that of Ashtamudi estuary. Robust cardinal teeth in the clam population of Kayamkulam estuary is the result of growing need for mechanical stability in shallow estuary. These are phenotypic

shifts to functional adaptations. The environmental characters of the estuary modify the dimensional characters in the organisms.

The present allometric study underpins the application of allometric data in the management of clam species *Paphia malabarica* and *Meretrix casta*. The application of allometric relationship between shell length and weight in *Paphia malabarica* and shell height and weight in *Meretrix casta* can be used as the most useful relation for stock assessment and management of these species.

Nucleotide sequence analysis in clams *Paphia malabarica*, morphotype of *Paphia malabarica* and *Meretrix casta* generated nucleotide sequences respectively with 895 bp, 862 bp, 787bp. All phylogenetic trees were supported by very high bootstrap values (60 % to 100%).The Neighbor joining (NJ), Maximum parsimony (MP) and Minimum Evolution (ME) trees are with same tree topology. All the tree analyses point to the presence of three principal groups. Analysis of nucleotide sequences of ITS1, 5.8S, ITS2 and 28S regions of nuclear ribosomal DNA of Venerid clams *Paphia malabarica* and *Meretrix casta* revealed that the ITS is species dependent and it can be utilised for species identification. The phylogenetic analyses of the nucleotide sequences confirmed that the sub families are monophyletic as their family Veneridae.

Even though the initial molecular analysis indicated the genetic difference between the *Paphia* populations of Ashtamudi estuary, further phylogenetic analyses ruled out the existence of genetic difference between them. The discrepancy between the morphological differences and genetic similarity can be attributed to the environmental factors influencing the morphology and thus resulting in phenotypic plasticity.

Organisation of Thesis

The thesis is organised in 10 chapters. Chapter 1 deals with the general introduction of the research work. It explains the research problem, hypothesis of the work, significance and objectives of the study. Chapter 2 depicts the study areas and sampling sites marked in the estuaries. Chapter 3 unravels the hydrological characters of clam beds in two estuaries during different seasons. Physico-chemical parameters of sediment in the clam beds of two estuaries are expounded in chapter 4. Chapter 5 delineates the density and distribution of clams *Paphia malabarica* and *Meretrix casta* in two estuaries. It also shows the correlation between the environmental and biological variables. Chapter 6 extricates the environmental variables exerting influence on the biological variables of two estuarine systems using the

Principal Component analysis (SPSS version 20). Morphometry and allometry of two clam species are analysed in chapter 7. Chapter 8 deciphers the nucleotide sequence (rDNA) of clams *Meretrix casta*, *Paphia malabarica* and its morphological variant. It also unveils the phylogenetic relationships in 21 taxa of Veneridae. Chapter 9 summarises the findings, conclusions and recommendations of the study. Chapter 10 lists all the references.

List of Significant publications produced from the study

Ampili, M. and Shiny Sreedhar, K. 2012. Nutritional Conditions: Condition Index, Percentage Edibility and Biochemical Constituents in *Paphia malabarica (Chemnitz).IJAFST, 3(1):1-19.*

Ampili, M. and Sreedhar, S.K. 2014. Molecular Phylogeny of Veneridae (Mollusca: Bivalvia) based on Nuclear Ribosomal Internal Transcribed Spacer Region. *International Journal of Molecular Biology*, 5 (1): 96-101.

Paper presented

Ampili M. and Shiny Sreedhar,K. 2012. Ecology of Clam Bed- Hydrographic conditions Influencing the Density and Distribution of Venerid Clam *Paphia malabarica* in two Lake Systems. *LAKE 2012*, National Conference on Conservation and Management of Wetland Ecosystems.